

Construction and Characterization of a Deletion Mutant Lacking *micF*, a Proposed Regulatory Gene for OmpF Synthesis in *Escherichia coli*

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A method is presented for the construction of a deletion mutant lacking the *micF* gene, which has been proposed to negatively regulate expression of the *ompF* gene. The method includes (i) construction of a temperature-sensitive plasmid containing a chromosomal fragment that carries both flanking regions of the *micF* gene but does not carry *micF* itself and (ii) replacement of the corresponding chromosomal domain with the fragment. The method is applicable to construction of a deletion mutant for any *Escherichia coli* chromosomal gene provided that it is dispensable. The *micF* deletion was confirmed by genetic and biochemical tests, including nucleotide sequence analysis. *ompF* expression in the *micF* deletion mutant thus constructed was normally regulated and was not enhanced. When *micF* was cloned into a high-copy-number plasmid it repressed *ompF* gene expression, whereas when cloned into a low-copy-number plasmid it did not. From these results, it is concluded that a single copy of the *micF* gene on the *E. coli* chromosome does not play a critical role in *ompF* gene expression.

The outer membrane of *Escherichia coli* contains two major porin proteins, OmpF and OmpC, that serve as channels for the passive diffusion of small hydrophilic molecules (25) and as constituents stabilizing the cell surface (26). These proteins are encoded by the *ompF* and *ompC* genes, respectively. Both genes have been cloned (19, 23), and their total nucleotide sequences have been determined (13, 20). These studies revealed high homology in the deduced amino acid sequences as well as the nucleotide sequences in the coding regions between *ompF* and *ompC* (20). On the other hand, the promoter structures of these genes are considerably different.

Although the functional and structural properties of the two proteins are similar, the expression of the genes is regulated in opposite directions by the medium osmolarity (15, 31). As the osmolarity increases, OmpF synthesis is depressed, with a concomitant increase in OmpC production. Studies with hybrid genes, such as the *ompF-lacZ* and *ompC-lacZ* fusions (9, 10), and chimeric genes between the *ompF* and *ompC* genes (17) revealed that the promoter region is primarily responsible for osmoregulation. Expression of *ompF* and *ompC* is also regulated by the *ompR* and *envZ* genes in the *ompB* operon (7, 11, 24, 30). The OmpR protein serves as a positive regulator for the *ompF* and *ompC* expression; the role of *envZ* is less clear.

Recently, Mizuno et al. (21, 22) found a third regulatory gene, *micF*, which codes for a small RNA molecule. This gene is located to the right upstream of *ompC*, and its transcription direction is opposite that of the *ompC* gene. *micF* gene expression is under the control of the *ompB* operon in the same manner as the *ompC* gene is. The primary structure of the *micF* RNA is complementary to the 5'-end region of the *ompF* mRNA, and when cloned into pBR322 the gene inhibits the production of the OmpF protein. Based on these facts, *micF* RNA was proposed to inhibit translation of the *ompF* mRNA by hybridizing with it

and to play a critical role in the osmoregulation of OmpF and OmpC synthesis.

For a critical study on the role of *micF* in expression of the *ompF* and *ompC* genes, we constructed a *micF* deletion mutant. The method includes (i) construction of a plasmid containing a chromosomal fragment that carries both flanking regions of the *micF* gene but does not carry the *micF* gene itself and (ii) replacement of the corresponding chromosomal domain with the fragment. *ompF* expression in the mutant was osmoregulated normally and was not enhanced, suggesting that one copy of the *micF* gene does not play a critical role in osmoregulation. We also examined the effect of the copy number of *micF* on *ompF* expression. Although when *micF* was cloned into a high-copy-number plasmid it inhibited OmpF synthesis, when it was cloned into a low-copy-number plasmid it did not. Based on these observations, the role of the *micF* gene is discussed.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. The *E. coli* K-12 strains, bacteriophages, and plasmids used in this work are listed in Table 1.

Media. Expression of the *ompF* and *ompC* genes was studied by using medium A supplemented with different concentrations of sucrose as described previously (15). The sensitivity of the cells to phage T4 was tested by cross-streaking on medium A with 15% (wt/vol) sucrose. Transformation experiments were carried out in 1% tryptone-0.5% yeast extract. When required, ampicillin, chloramphenicol, and kanamycin were added at concentrations of 50, 25, and 30 µg/ml, respectively. For solid cultivation, the medium was supplemented with 1.5% agar.

Cell envelope preparation and polyacrylamide gel electrophoresis. Preparation of cell envelopes, solubilization with Triton X-100, and urea-sodium dodecyl sulfate-polyacrylamide gel electrophoresis were carried out as described previously (24).

DNA techniques. Restriction endonucleases, bacteriophage T4 ligase, exonuclease *Bal* 31, S1 nuclease, *Hind*III

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TABLE 1. Bacteria, bacteriophages, and plasmids

Strain, phage, or plasmid	Relevant properties	Reference or source
<i>E. coli</i> K-12		
MC4100	F ⁻ Δ lacU169 araD rpsL relA thi fibB	4
MH760 recA	F ⁻ Δ lacU169 araD rpsL relA thi fibB ompR472 gyrA recA	12
HO201	F ⁻ thi rel rpsL mal λ^+	32
HO201 ompC	ompC derivative of HO201	27
YO160 recA	F ⁻ thi rel rpsL ompC envZ Φ (ompC-lacZ) recA	17
CE1036 recA	F ⁻ thi lacY galK mtl xyl ara rpsL supE ompC T6 ^r recA	17
SM3001	MC4100 Δ micF1	This study
SM3002	Δ micF1 transductant of HO201; donor, SM3001	This study
Bacteriophages		
Tu1b	Receptor; OmpC and lipopolysaccharide	6
P1kc	Used for generalized transduction	Our laboratory stock
Plasmids		
pMAN002	Cm ^r ; vector, pACYC184; cloned gene, ompC micF	17
pMAN006	Ap ^r ; vector, pKEN403; cloned gene, ompC micF	17
pMAN005	Same as pMAN006 except for direction of the cloned fragment	This study
pSY343	Km ^r	33
pEL3	Ap ^r ; temperature-sensitive replicon	1
pKM004	Ap ^r ; vector, pBR322; cloned gene, lpp promoter-controlled lacZ-lacY operon	21
Plasmid III	Ap ^r ; vector, pKM005; cloned gene, micF	22
pKEN403	Ap ^r Km ^r ; replication origin derived from pSC101	K. Nakamura
pACYC184	Cm ^r Tc ^r	5
pBR322	Ap ^r Tc ^r	2

linker (dCAAGCTTG), and *Bam*HI linker (dCGGATCCG) were obtained from Takara Shuzo Co. The conditions used for digestion with these nucleases were those proposed by the manufacturer. [γ -³²P]ATP labeling at the 5' ends of DNA fragments for hybridization and sequencing and DNA sequencing were carried out as described by Maxam and Gilbert (18). Southern transfer was carried out as described previously (17), and the blot was hybridized with a ³²P-labeled probe (10⁶ cpm/ml) at 37°C in the presence of a deionized solution of 50% formamide-5 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]). Other general manipulations such as preparation of plasmid and chromosomal DNAs, digestion with restriction endonucleases, ligation, and transformation were performed as described previously (17).

RESULTS

Construction of plasmid pMAN036. Deletion of the *micF* region from the chromosome was carried out in two steps: (i) construction of a plasmid containing a chromosomal DNA fragment that carries both flanking regions of the *micF* gene but does not carry the *micF* gene itself, and (ii) replacement

of the corresponding chromosomal domain with the fragment. For this purpose, pMAN036 was first constructed. The detailed procedure for its construction is shown in Fig. 1. Structures of the individual plasmids shown in Fig. 1 were confirmed by DNA restriction analysis.

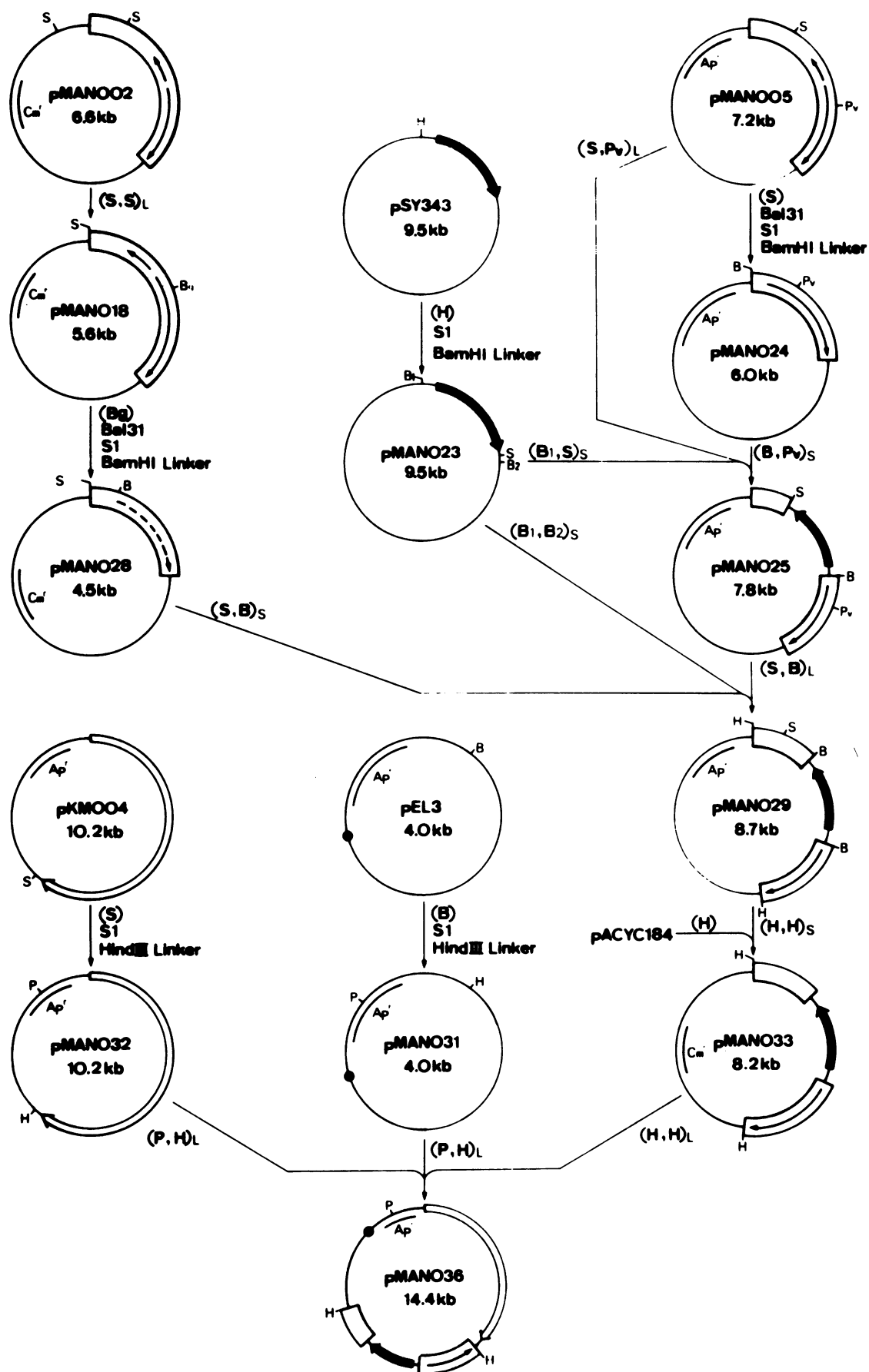
pMAN005, pMAN002, and pSY343 were used as starting plasmids. Deletion by *Bal* 31 digestion was performed for pMAN005 and pMAN018 to construct pMAN024 and pMAN028, respectively. For pMAN024, digestion was initiated at the *Sal*I site located about 550 nucleotides downstream from the possible *micF* termination signal (22) and reached the *micF* promoter region. For pMAN028, digestion was initiated at the *Bgl*II site located near the promoter-proximal end of *ompC* and reached the promoter-distal end of *micF*. The extent of the deletion was determined by size analysis of the *Bam*HI-*Bgl*II fragment for pMAN024 and of the *Sal*I-*Bam*HI fragment for pMAN028.

From these plasmids was constructed pMAN029. Since we wanted to construct other plasmids together with pMAN029, the process (Fig. 1) was somewhat complex. Plasmid pMAN029 carries both flanking regions of the *micF* gene but carries the kanamycin resistance (Km^r) gene in place of *micF*. The structure of the plasmid was confirmed by DNA restriction analyses and the presence of both the Km^r and *ompC* genes. The vector domain of pMAN029 was then replaced by that of pACYC184 carrying the chloramphenicol resistance (Cm^r) gene to facilitate the selection of pMAN036. Finally, from pMAN033, pKM004, and pEL3 was constructed pMAN036. This plasmid carries (i) the *ompC-micF* region of the chromosomal DNA in which the *micF* gene is replaced by the Km^r gene, (ii) a temperature-sensitive replicon, and (iii) the *lpp* promoter-controlled *lacZ-lacY* operon. All of these characteristics except the absence of *micF* were confirmed biochemically. The structure of pMAN036 was also confirmed by DNA restriction analysis of the entire plasmid.

Construction of a chromosomal mutant with *micF* deletion. A *micF* deletion mutant was constructed by replacing the *micF-ompC* region of the chromosome with the Km^r-*ompC* region of pMAN036 via homologous recombination (Fig. 2). Use of the temperature-sensitive replicon-carrying plasmid enabled us to isolate transformants in which the plasmid had integrated into the chromosome. Use of the Km^r and *lac* genes arranged as in pMAN036 facilitated selection of the *micF* deletion mutant.

E. coli MC4100 (Δ lac) was transformed with pMAN036 and grown at 30°C for 2 h. It was further incubated on lactose-MacConkey plates containing kanamycin (30 μ g/ml) at 42°C overnight. Since pMAN036 has the temperature-sensitive replicon, the transformants should appear as Km^r colonies at 42°C only after incorporation of the plasmid into the chromosome (1, 8). Most of the transformants appeared as red colonies (Km^r Lac⁺), indicating that the plasmid had been integrated into the chromosome by a single crossover at either region A or B (Fig. 2). About 1% of the transformants appeared as white colonies (Km^r Lac⁻). Provided that the events shown in Fig. 2 did take place in the transformants, they are the most probable candidates for chromosomal mutants carrying the *micF* deletion.

Determination of the genomic structure of SM3001, a *micF* deletion mutant. Three experiments were performed to confirm the gene structure of the relevant region of strain SM3001, one of the presumed *micF* deletion mutants. First, P1 transduction was carried out with strain SM3001 as the donor and strain HO201 *ompC* as the recipient. All of the 300 Km^r transductants isolated were OmpC⁺, indicating that the



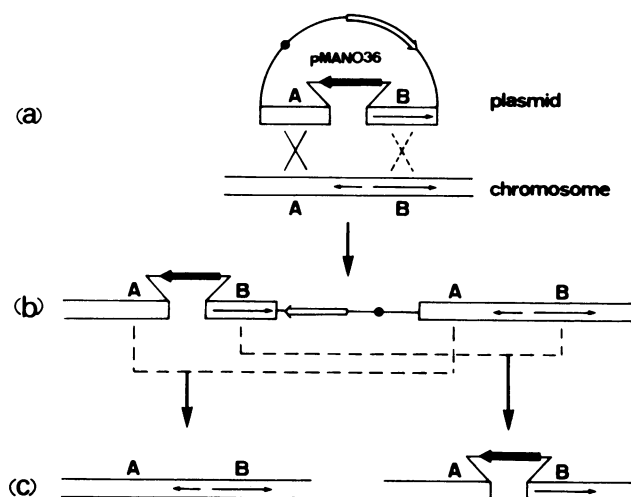


FIG. 2. Replacement of the *micF-ompC* region of the chromosome with the *micF*-deleted corresponding region in pMAN036. Strain MC4100, a Δ *lac* strain, was transformed with pMAN036 (Fig. 1) and incubated at 42°C on lactose-MacConkey plates containing kanamycin. Plasmid integration into the chromosome by homologous recombination takes place at either region A or region B (a). Transformants having the plasmid in the chromosome are Km^r Lac^+ . In the case of recombination at region A, subsequent plasmid segregation from the chromosome by homologous recombination takes place at either of the regions indicated by dotted lines in (b). This results in one of the chromosomal structures shown in (c). The segregated plasmids are lost in the cells because of the inability to replicate autonomously at 42°C. Recombination for plasmid segregation at region A gives the same chromosomal structure as that of an untransformed cell, whereas recombination at region B results in replacement of the *micF* gene with the Km^r gene. This *micF* deletion mutant is detected as a white colony (Lac^-) on a lactose-MacConkey plate containing kanamycin. When recombination for plasmid integration takes place at region B (a), the subsequent process is principally the same. The symbols used are described in the legend to Fig. 1.

Km^r gene is very closely linked to the *ompC* gene on the chromosome.

In the second experiment, the chromosomal DNA of strain SM3001 was analyzed by Southern hybridization to confirm the absence of the *micF* gene. When the 2.7-kilobase (kb) *Hind*III fragment carrying the *ompC-micF* region isolated from pMAN002 was used as a probe, hybridization with the *Hind*III digest of the wild-type chromosomal DNA took place at the position of 2.7 kb as expected (Fig. 3A, lane 2). On the other hand, the probe hybridized with the *Hind*III digest of the strain SM3001 chromosomal DNA at the position of 4.2 kb (Fig. 3A, lane 3). This size was the same as that of the *Hind*III fragment of pMAN033 that carries the *ompC* region in which the *micF* gene has been replaced by the Km^r gene. No other bands were observed on Southern blotting. Hybridization analysis was also performed with a

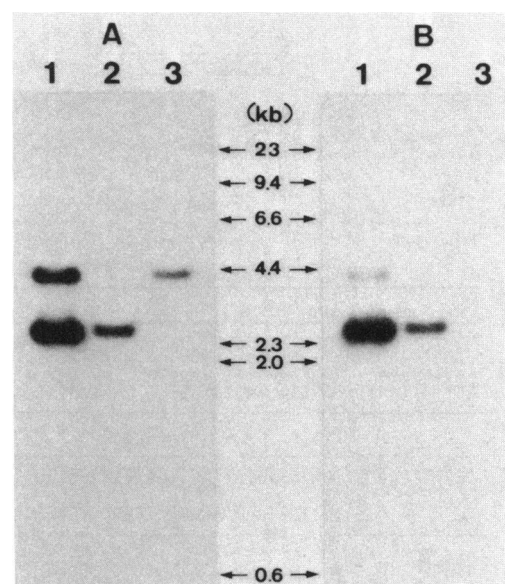


FIG. 3. Southern blot analyses of the *micF* chromosomal deletion. A mixture of pMAN002 and pMAN033 (lanes 1) and chromosomal DNA from strains MC4100 (lanes 2) and SM3001 (lanes 3) was digested with *Hind*III and analyzed as described in the text. The 2.7-kb fragment containing the *micF-ompC* region from pMAN002 (A) and the 120-bp fragment containing a larger part of the *micF* gene (Fig. 4) (B) were used as probes. The *Hind*III fragments of bacteriophage λ DNA were used for standardization of the base length.

120-base-pair (bp) fragment carrying a larger part of the *micF* gene. The probe hybridized with the *Hind*III digest of strain MC4100 chromosomal DNA at the position of 2.7 kb, but no significant hybridization was observed with the *Hind*III digest of strain SM3001 chromosomal DNA (Fig. 3B). Weak hybridization took place at the position of 4.2 kb. As revealed later by DNA sequencing analysis, the 4.2-kb *Hind*III fragment of strain SM3001 still possessed a small portion of the promoter-distal end of the *micF* gene that shares 17 nucleotides with the 120-bp probe (Fig. 4). Taken together, these results demonstrate that (i) the *micF* gene is almost fully deleted and replaced by the Km^r gene in the mutant strain SM3001 chromosome and (ii) the wild-type *E. coli* chromosome possesses only one copy of *micF*.

In the third experiment, the relevant region of strain SM3001 chromosomal DNA was recloned into a plasmid, and the nucleotide sequence was determined. The chromosomal DNA of strain SM3001 was digested with *Hind*III, and the fragments that migrated to the 4.2-kb region on an agarose gel were cloned into the *Hind*III site of pACYC184, which in turn was used to transform strain CE1036 *recA*. Transformants were first selected for Cm^r Km^r . All four transformants thus selected were sensitive to phage TuIb,

FIG. 1. Construction of plasmid pMAN036. □, Chromosomal DNA of the *micF-ompC* region. The short and long arrows in the open boxes indicate the coding region and the direction of transcription of *micF* and *ompC*, respectively; broken arrow represents the nonfunctioning *ompC* gene, the upstream region of which has been deleted. ⇨ and ⇩, Coding region and direction of transcription of *lacZ-lacY* and Km^r , respectively; —, vector plasmid DNA; ●, temperature-sensitive replicon. The restriction endonucleases used are shown in parentheses (abbreviations: B, *Bam*HI; Bg, *Bgl*II; H, *Hind*III; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I). Cleavage sites are shown. When one enzyme cleaves at more than one site in a plasmid, the sites are distinguished with numbers, e.g., B₁ and B₂. S and L outside parentheses denote small and large fragments, respectively, formed as a result of digestion with the endonuclease. All plasmids were transferred into strain YO160 *recA*, except for pMAN032, pMAN035, and pMAN036, which were transferred into strain MH760 *recA*. Antibiotic resistance genes and plasmid sizes are also indicated.

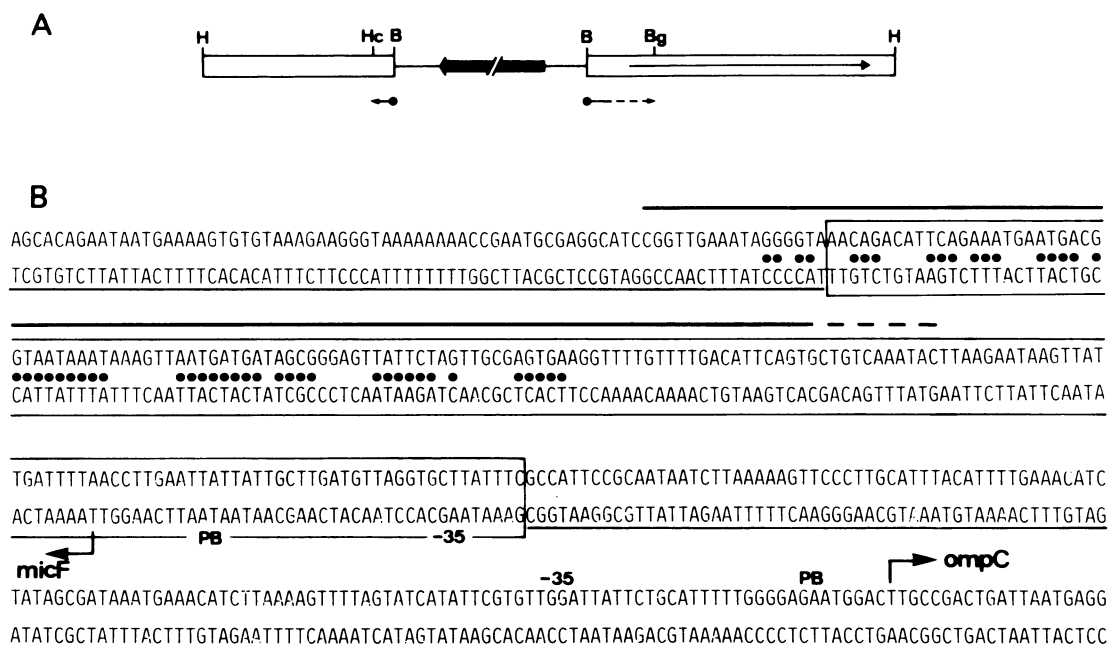


FIG. 4. DNA sequence analysis of the chromosomal *micF* deletion. (A) Strategy for nucleotide sequencing. The *micF-ompC* region on the mutant chromosome which was cloned into pMAN042 is shown. The symbols and abbreviations used are described in the legend to Fig. 1. Hc, *HincII*. Small arrows outside the box indicate fragments used for sequencing, with the position of the ^{32}P -labeled 5' end indicated (●). The broken region of an arrow indicates that the sequence of that region was not determined. (B) Nucleotide sequence around the *micF-ompC* region. The nucleotide sequences determined are underlined. The *micF* region that was deleted and replaced by the *Km^r* gene-carrying fragment is boxed. Dots between the two strands indicate sequences complementary to *ompF* mRNA. Arrows, Transcriptional initiation sites for *micF* and *ompC* (22). Pribnow boxes (PB) and -35 regions for the *micF* and *ompC* promoters are also indicated (22). A heavy line above the sequences indicates the 120-bp fragment used as a probe for the hybridization analysis (Fig. 3). This fragment was kindly prepared by T. Mizuno. The broken line indicates that the exact location of the terminus was not determined.

which requires the OmpC protein as a receptor. The restriction map of the 4.2-kb *HindIII* fragment thus cloned was the same as that of pMAN036 (data not shown). From these results we conclude that the strain SM3001 chromosome indeed carried the 4.2-kb *HindIII* fragment of pMAN036. One of the plasmids, pMAN042, was used for DNA sequencing to confirm deletion of the *micF* gene directly. The *Bam*HI-*Bam*HI subfragment of the 4.2-kb fragment, which originated in the *Km^r* vector plasmid, was removed, and the DNA sequences of the 90-bp *Hinc*II-*Bam*HI and 310-bp *Bam*HI-*Bgl*II subfragments were determined (Fig. 4A). Comparison with the known DNA sequences revealed that the *Hinc*II-*Bam*HI fragment represents sequences downstream from *micF* and the *Bam*HI-*Bgl*II fragment represents the right upstream region from the *ompC* promoter (Fig. 4B), indicating that the promoter region and the region complementary to the *ompF* mRNA of the *micF* gene were almost fully deleted. From all the evidence described above, we concluded that strain SM3001 is a *micF* deletion mutant.

Expression of *ompF* and *ompC* in the *micF* deletion mutant. The *micF* gene codes for a small RNA molecule whose primary structure is complementary to the upstream end of *ompF* mRNA, and the *micF* RNA was proposed to inhibit the translation of the *ompF* mRNA (22). It was also proposed that inhibition by the *micF* RNA is responsible for osmoregulation of *ompF* gene expression. It was therefore expected that the *micF* deletion would cause deregulation of *ompF* expression. Contrary to this expectation, *ompF* gene expression in the *micF* mutant was still osmoregulated normally and was not enhanced (Fig. 5). In fact, expression was slightly repressed. Also unexpectedly, *ompC* gene ex-

pression was significantly enhanced, especially at low osmolarity, although it was still osmoregulated. The enhancement of *ompC* gene expression with some repression of *ompF* gene expression in the *micF* mutant will be discussed below.

In any event, it is clear that osmoregulation of *ompF* gene expression was maintained in the *micF* mutant and that the *micF* deletion did not enhance OmpF synthesis. We conclude, therefore, that the presence of the *micF* gene is not crucial for osmoregulation of OmpF synthesis in wild-type *E. coli*.

Effect of *micF* copy number on expression of *ompF*. The *micF* gene cloned into a high-copy-number plasmid represses *ompF* gene expression (22). On the other hand, the present work suggests that a single copy of the *micF* gene on the chromosome does not repress *ompF* gene expression.

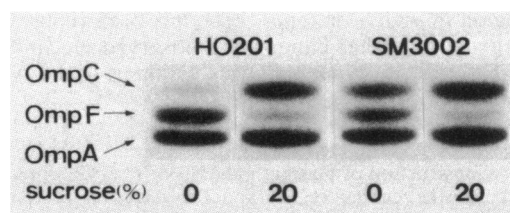


FIG. 5. Expression of *ompF* and *ompC* in the *micF* deletion mutant. Strains HO201 (wild-type) and SM3002 ($\Delta micF$) were grown with the indicated concentrations (wt/vol) of sucrose. Triton X-100-insoluble fractions (25 μg of protein) were prepared from cell envelopes and analyzed on polyacrylamide gels. The positions of OmpC, OmpF, and OmpA are indicated.

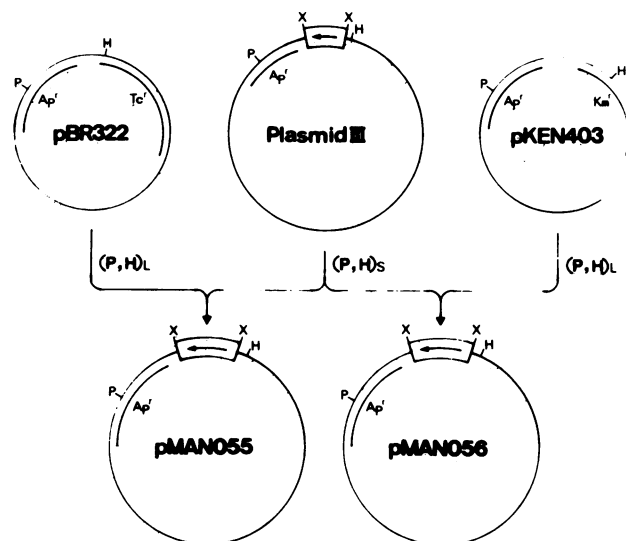


FIG. 6. Construction of *micF* gene-carrying plasmids pMAN055 and pMAN056. In plasmid III (22), the *micF*-carrying CX28 fragment (300 bp) is inserted between the *Xba*I sites (X). The arrow in the box represents *micF*. The 1.0-kb *Pst*I-*Hind*III fragment from plasmid III was ligated with the 3.6-kb *Pst*I-*Hind*III vector fragment from pBR322 or the 3.8-kb *Pst*I-*Hind*III vector fragment from pKEN403 to construct pMAN055 and pMAN056, respectively. The structures of the two plasmids thus constructed were confirmed by DNA restriction analysis. See the legend to Fig. 1 for other abbreviations.

We therefore examined the effect of the *micF* gene cloned into plasmids at different copy numbers. pBR322 and pKEN403 were used as high- and low-copy-number plasmid vectors, respectively. The *micF* gene-carrying fragment was prepared from plasmid III (22) and cloned into pBR322 and pKEN403 to construct pMAN055 and pMAN056, respectively (Fig. 6). The ratio of the copy number of pMAN055 to that of pMAN056 in strain MC4100 was about 5 to 1 (data not shown). Strain MC4100(pMAN055) lost the ability to produce OmpF (Fig. 7, lane 1), whereas in MC4100(pMAN056), no significant repression of OmpF synthesis was observed (Fig. 7, lane 2). The results were the same irrespective of the osmolarity of the medium. The copy number per genome equivalent of pMAN056 was assumed to be about six, since the vector domain of the plasmid was derived from pSC101 (3). It is reasonable to assume, therefore, that a single copy of the *micF* gene on the *E. coli* chromosome is insufficient to repress *ompF* gene expression significantly.

DISCUSSION

Techniques of gene replacement between plasmids and chromosomes via homologous recombination have been developed (8, 14, 28). The techniques require (i) a plasmid that cannot replicate extrachromosomally and (ii) a simple selection method for the plasmid integrate and also one for segregating the plasmid from the plasmid integrate (Fig. 2). A plasmid integrate is a strain in which a direct nontandem duplicate of bacterial sequences separated by vector sequences is integrated into the chromosome via homologous recombination.

The method of gene replacement we have developed in this work is unique in the following points. The use of a plasmid carrying the *Km*^r and *lac* genes is the major advantage of the present method, especially for the construction of

a deletion mutant, since a deletion mutant can be efficiently obtained by one-step selection for *Km*^r *Lac*⁻ on MacConkey plates without knowing the phenotype caused by the mutation. The use of a plasmid carrying the temperature-sensitive replicon that cannot replicate at 42°C is another advantage over the use of a ColEI-like plasmid (8), whose replication depends on the *polA* gene (16), because in the latter case the construction strain cannot be transformed with a plasmid that is unable to replicate in the *polA* background. Our methodology is applicable to the isolation of mutants carrying deletions of any *E. coli* chromosomal gene provided that it is dispensable.

We constructed a *micF* deletion mutant by the procedure described above. The deletion was confirmed by various genetic and biochemical tests, including nucleotide sequence analysis. We also confirmed that the wild-type *E. coli* chromosome, from which the *micF* deletion mutant was constructed, possesses only one copy of the *micF* gene. Contrary to expectation on the basis of the proposed function of *micF*, *ompF* expression was not enhanced and was still osmoregulated normally in the *micF* mutant, indicating that the *micF* gene on the chromosome does not play a critical role in osmoregulation.

It should also be noted that in the *micF* deletion mutant *ompC* gene expression was enhanced, with slight repression of *ompF* gene expression. The promoter regions of *ompC* and *micF* are located next to each other on the *E. coli* chromosome (22). It can be assumed, therefore, that both promoters compete with each other for an RNA polymerase molecule, so that the deletion of one of them enhances expression of the other. This view was supported by recent analyses of expression of the two genes (unpublished data). The decrease in *ompF* expression in the *micF* deletion mutant may be a result of the high expression of *ompC*.

It was reported that the *micF* gene cloned in a high-copy-number plasmid repressed *ompF* expression (22). This result, together with the present work, suggests that the gene dosage would be crucial to the functioning of the *micF* RNA as a repressor. This view was supported by the results presented in Fig. 7, where it can be seen that the *micF* gene cloned in the low-copy-number plasmid did not repress *ompF* gene expression. Taking all the results together, we conclude that although the *micF* gene has the potential to repress *ompF* gene expression and osmoregulation as reported previously (21, 22), one copy of it on the chromosome is insufficient for it to play a critical role in osmoregulation under the conditions we employed in this work.

Recently, Schnaitman and McDonald obtained a deletion mutant lacking the *micF-ompC* region (29). OmpF synthesis in the mutant was partially constitutive; OmpF synthesis is significant in high-osmolarity media. The *micF* deletion

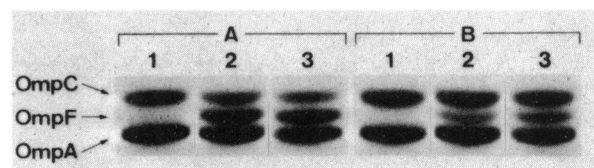


FIG. 7. Effect of the copy number of *micF* on expression of *ompF*. Strains MC4100(pMAN055) (lanes 1), MC4100(pMAN056) (lanes 2), and MC4100 (lanes 3) were grown in (A) medium A and (B) medium A supplemented with 8% (wt/vol) sucrose. Triton X-100-insoluble fractions (25 µg of protein) were prepared from cell envelopes and analyzed on polyacrylamide gels. The positions of OmpC, OmpF, and OmpA are indicated.

mutant we used in the present study was *ompC*⁺. We are presently studying the effects of *ompC* on *micF* function.

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